PROTOZOAN SYMBIONTS FROM THE ANEMONE *Bunodosoma cavernata* FROM GALVESTON ISLAND, TEXAS

by

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Hargitt (1904), in his classic paper on the medusae of the Woods Hole region, noted the susceptibility of medusoid forms to various protozoan parasites. He stated that "when working upon the regeneration of medusae I found several species of Protozoa very closely associated with them and, under the limitations of the aquaria, often exceedingly troublesome, seriously interfering with the progress of the experiments. This suggested the possibility of a parasitic relation." Unfortunately Hargitt made no mention of the taxa of Protozoa involved. Studies regarding protozoan infestations of Cnidaria have been few. Other than the passing references to protozoan infestations by Hargitt (1904) and Kudo (1966) there have been no intensive studies of protozoan parasites or symbionts of marine Cnidaria.

The anemone *Bunodosoma cavernata* is a common inhabitant of the Texas coast and cursory observation revealed a large complement of protozoan associates. These included *Saprodinium, Euplotes, Paraeuplotes, Vorticella, Cohnilembus, Anophrys, Uronema* and *Vahlkampfia.*

Approximately forty anemones were collected from jetty rocks on Galveston Island on 3 November 1970. Note was made within one day of protozoans found on the external surfaces, in the coelenteron and in surrounding sea water. The anemones were kept until 7 December 1970 under varying conditions and bi-weekly note was made of the associated protozoan fauna.

One group of six anemones was placed in a 29-gallon aquarium in which the salinity was maintained at 28 g/kg and the temperature ranged from 15 to 18°C. These anemones were considered "normal." No attempt was made to construct a facsimile of the Galveston littoral environment from which the anemones were removed. Other groups of three to eight anemones were kept in 1.5-gallon aquaria (salinity 28 g/kg, temp. 18°C) and in 12-inch finger bowls wherein salinity and temperature were varied. For one group (GP 1) the salinity was allowed to rise by evaporation from 20 g/kg to 40 g/kg over a 12-day period. In a second group (GP 2) the sea water was flushed out and

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replaced with clean sea water of the same salinity every 3 days for a 3-week interval. In the third group (GP 3), the salinity was constantly maintained at 24 g/kg for 4 weeks. All three groups were maintained at 18°C. In a fourth group temperature was maintained at 24°C. Salinity was routinely monitored on a daily basis with a Goldberg refractometer. Microscopy examinations were made every 2 to 4 days using wet mounts of anemone mucus, coelenteron contents, tissue squashes and the external medium.

Protozoans found as epizoites or symbionts on the anemones as well as site of infection and relative abundance are listed in Table 1.

Table 1.

Protozoa Found in Association with Anemones after Two Weeks in the Laboratory

Protozoan	Site of Infection			Size Range (microns)
	Wounds	Coelenteron	Mucus Coat	
Vahlkampfia	XXX	XX	XX	7-30
Saprodinium	XX	X	X	21-25
Euplotes	XXX	XXX	XXX	70-140
Paraeuplotes	X	X	X	50-80
Paramecium			X	100-150
Vorticella	XX	XX	XX	c.150
Cohnilembus	X	X	XX	30-80
Anophrys	XX	XX	XX	60-90
Uronema	X	X	X	20-40

X indicates rarity; XX indicates frequent occurrence; XXX indicates dominant organisms numerically.

These data were taken 2 weeks after the anemones were removed from the natural environment and introduced into the laboratory. Initially when first placed in aquaria all Protozoa found in association with the anemones occurred in very low numbers when compared to the situation after 2 weeks. Protozoa in habitat sea water included very sparse populations of *Euplotes, Paramecium* and *Vorticella*. The normal anemones maintained a numerically smaller symbiont population than those in the finger bowls. Those in the 1.5-gallon aquaria had a "normal" symbiont complement also. In GP 2, where the water was flushed out and replaced periodically, the anemones retained the normal fauna

and did not support large populations of any species of protozoan. In the group maintained at 24°C the anemones became moribund after two weeks and developed extremely large populations of ciliates, especially *Euplotes*, *Anophrys* and *Saprodinium*, as well as large numbers of the naegleriid amoeba *Vahlkampfia*. In addition to being found in necrotic and normal anemone tissues this amoeba was also found in large numbers in the fouled medium. Both cysts and trophozoites of *Vahlkampfia* were extremely numerous at sites of necrosis. In group 3 (GP 3), wherein conditions were constant, the anemones survived up to 4 weeks at which time they became moribund and died. The same situation prevailed here as with respect to GP 2. In GP 1 increased salinity had no apparent effect on protozoan numbers although individual *Euplotes* showed considerable size increase (up to 250 μ in length). Size ranges of all associative Protozoa encountered are given in Table 1.

All anemones within the finger bowls died within 5 weeks. In all cases the anemones were infiltrated by large numbers of bacteria and large populations of *Euplotes* and *Vahlkampfia*. Approximately half the *Euplotes* in association with necrotic anemone tissue were in the process of conjugation.

Three morphological types of *Vahlkampfia* were observed. Most amoebae were 7–10 μ in length and formed only one broad, fan-shaped pseudopod which, moreover, consisted mainly of a large hyaline area. Formation of a new pseudopod was preceded by flooding the old pseudopod with granular endoplasm and the formation of a new hyaline bulge. Rate of pseudopod formation was hastened by the heat of a substage illuminator. The second observed trophozoite stage was considerably larger (25–30 μ) and had numerous psuedopodia. Their morphology approached that of the former type after being on a microscope slide (above an illuminator) for several minutes. These larger amoebae underwent division and "reverted" to the smaller, monopseudopodial type. On two occasions a nucleus (2–3 μ in diameter) with a large endosome was observed in the large amoebic variant. Large numbers of *Vahlkampfia* cysts were found in decaying and moribund anemones as well as in putrefying media. After being on a microscope slide for about 5 minutes, above a substage illuminator, these cysts released one amoeba each. Cysts were rounded, considerably flattened, had wrinkled surfaces and were between 5–9 μ in diameter. *Vahlkampfia* invades anemone tissue and will emerge from tissue fragments when heated by substage illumination for a few minutes. The amoebae can be demonstrated in both normal and necrotic tissue, the greatest concentration of amoebae occurring in necrotic tissues with a large bacterial population. The amoebae were observed to ingest bacteria, indicating that greater degree of association with necrotic tissue may be due to greater availability of food organisms in necrotic as opposed to healthy tissue. Other Protozoa, especially *Eu-*

plotes, showed greater abundance where there was high bacterial density.

Vahlkampfia and at least a few of the ciliates (*Euplotes, Paraeuplotes, Saprodinium* and *Vorticella*) are not obligate symbionts. Naegleriid amoebae are known as facultative parasites from a wide variety of organisms including rodents (Wilson et al. 1967), molluscs (Hogue 1921 and Richards 1970), insects (Page 1970) and man (Cerva and Novak 1968 and Duma et al. 1969). Apparently amoebae of this group can be demonstrated to be symbiotic with most any metazoan. Although *Naegleria gruberi* is the only naegleriid amoeba definitely known to be a causative agent in human amoebic meningoencephalitis (Duma et al. 1969 and Cerva 1970), the remaining naegleriids cannot be discounted as potential pathogens. Wilson et al. (1967) demonstrated that *Vahlkampfia* as well as many other genera of amoebae can cause systemic amoebiasis in rodents following hypodermic inoculation. Available data indicate that *Vahlkampfia*, and at least some of the ciliates in this study feed on broken tissue and associated bacterial flora. The external mucus coat or pellicle of the anemone on normal anemones has a relatively large bacterial population and this may account for the establishment of larger populations of Protozoa in this region when compared to other anatomical regions of the anemone.

The hymenostomid genera *Cohnilembus* and *Anophrys* are interesting in that they are known to be intestinal symbionts of echinoids (Kudo 1966). These two genera and *Uronema* predominantly occur in the coelenteron and mucus coat. *Paraeuplotes*, found here on anemones, is also a known epizoite of corals (Kudo 1966).

In the normal littoral habitat the anemones are periodically exposed to the air during tidal cycles and there is considerable scouring of the anemone surface as well as drastic short term salinity and temperature changes in the immediate environment of the anemone. These environmental factors probably serve to control bacterial and protozoan populations epizootic on the anemones. Conversely the mucus sheath of the anemone, which serves for attachment of protective shell fragments and other debris to the anemone surfaces, may prevent desiccation at low tide and may act as a suitable substrate for the establishment of epizoites. When exposed the anemones close the oral apparatus, trapping sea water and any associated microfauna possibly allowing survival of some associative forms. It remains to be determined whether or not any of these symbionts or epizoites are dependent on the anemone for life cycle completion.

Euplotes is by far the most abundant of all the ciliates encountered and conjugation is a commonplace phenomenon especially when putrefaction is well advanced. It is possible that death of the anemone enables or induces conjugation in *Euplotes*.

Anemones of the littoral zone support an extensive protozoan fauna. Although none of the Protozoa involved are definitely known to be obligate symbionts there is a distinct association. Most of the forms are facultative symbionts, especially *Vahlkampfia* and *Euplotes*. *Vahlkampfia* invasion of anemone tissues can to some extent be associated with necrotic tissue changes and increased bacterial populations. Most probably bacteria initiate the necrosis and the large bacterial populations allow for increase in the amoeba population. No causal relationship has been established.

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